5. Witte, H., Neukirchen, D., and Bradke, F. (2008). Microtubule stabilization specifies initial neuronal polarization. J. Cell Biol. *180*, 619–632.
6. Arimura, N., and Kaibuchi, K. (2007). Neuronal polarity: from extracellular signals to intracellular mechanisms. Nat. Rev. Neurosci. *8*, 194–205.
7. De Anda, F., Pollarolo, G., Da Silva, J., Camoletto, P., Feiguin, F., and Dotti, C. (2005). Centrosome localization determines neuronal polarity. Nature *436*, 704–708.
8. Collin, L., Schlessinger, K., and Hall, A. (2007). APC nuclear membrane association and microtubule polarity. Biol. Cell *100*, 243–252.
9. Vitre, B., Coquelle, F., Heichette, C., Garnier, C., Chrétien, D., and Arnal, I. (2008). EB1 regulates microtubule dynamics and tubulin sheet closure *in vitro*. Nat. Cell Biol. *10*, 415–421.
10. Bartolini, F., Moseley, J.B., Schmoranzer, J., Cassimeris, L., Goode, B.L., and Gundersen, G.G. (2008). The formin mDia2 stabilizes microtubules independently of its actin nucleation activity. J. Cell Biol. *181*, 523–536.
11. Akum, B.F., Chen, M., Gunderson, S.I., Riefler, G.M., Scerri-Hansen, M.M., and Firestein, B.L. (2004). Cypin regulates dendrite patterning in hippocampal neurons by promoting microtubule assembly. Nat. Neurosci. *7*, 145–152.
12. Fukata, Y., Itoh, T.J., Kimura, T., Ménager, C., Nishimura, T., Shiromizu, T., Watanabe, H., Inagaki, N., Iwamatsu, A., Hotani, H., *et al.* (2002). CRMP-2 binds to tubulin heterodimers to promote microtubule assembly. Nat. Cell Biol. *4*, 583–591.
13. Burack, M.A., Silverman, M.A., and Banker, G.A. (2000). The role of selective transport in neuronal protein sorting. Neuron *26*, 465–472.
14. Sharp, D.J., Yu, W., Ferhat, L., Kuriyama, R., Rueger, D.C., and Baas, P.W. (1997). Identification of a microtubule-associated motor protein essential for dendritic differentiation. J. Cell Biol. *138*, 833–843.
15. Myers, K.A., and Baas, P.W. (2007). Kinesin-5 regulates the growth of the axon by acting as a brake on its microtubule array. J. Cell Biol. *178*, 1081–1091.
16. Ahmad, F.J., He, Y., Myers, K.A., Hasaka, T.P., Francis, F., Black, M.M., and Baas, P.W. (2006). Effects of dynactin disruption and dynein depletion on axonal microtubules. Traffic *7*, 524–537.
17. Dehmelt, L., Nalbant, P., Steffen, W., and Halpain, S. (2006). A microtubule-based, dynein-dependent force induces local cell protrusions: Implications for neurite initiation. Brain Cell Biol. *35*, 39–56.
18. Myers, K.A., He, Y., Hasaka, T.P., and Baas, P.W. (2006). Microtubule transport in the axon: Re-thinking a potential role for the actin cytoskeleton. The Neuroscientist *12*, 107–118.
19. Takahashi, D., Yu, W., Baas, P.W., Kawai-Hirai, R., and Hayashi, K. (2007). Rearrangement of microtubule polarity orientation during conversion of dendrites to axons in cultured pyramidal neurons. Cell Motil. Cytoskeleton *64*, 347–359.
20. Dent, E., Kwiatkowski, A., Mebane, L., Philippar, U., Barzik, M., Rubinson, D., Gupton, S., Van Veen, J.E., Furman, C., Zhang, J., *et al.* (2007). Filopodia are required for cortical neurite initiation. Nat. Cell Biol. *9*, 1347–1359.

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Plastid Evolution: Remnant Algal Genes in Ciliates

Determination of the number of times that plastids have been gained and lost during eukaryotic evolution has proven difficult. A recent study has uncovered what could be the molecular signature of a photosynthetic ancestry for an important plastid-lacking lineage of microbial eukaryotes — the ciliates.

John M. Archibald

Ask a ciliate biologist what their organism of choice can tell us about the evolution of eukaryotic photosynthesis and the answer is likely to be "what are you talking about?". This would be a reasonable response. After all, ciliates have been intensely studied for decades, being famous for their nuclear dualism [1] and important contributions to the field of molecular biology (e.g., telomerase was discovered in ciliates [2]): they are definitely *not* photosynthetic. Nor is there any evidence that they harbor a remnant plastid, as seen in the malaria parasite *Plasmodium* [3]. Far from being passive sunbathers, ciliates play the role of voracious predator in diverse microbial ecosystems ranging from the anaerobic guts of animals to the open ocean. Nevertheless, by virtue of their position on the eukaryotic tree, ciliates are part of a long-standing argument over the tempo and mode of plastid evolution, specifically the question of how often such organelles have been gained and lost during the history of eukaryotes. In a recent issue of *Current Biology*, Reyes-Prieto *et al.* [4] presented phylogenomic evidence suggesting that, despite their current lifestyle, ciliates may have evolved from photosynthetic ancestors, an observation that is certain to heat up the simmering debate over plastid evolution.

The photosynthetic organelles of algae and plants — plastids — evolved from cyanobacteria by endosymbiosis. It is widely believed that 'primary' plastids evolved only once, probably in the common ancestor of red, green and glaucophyte algae [5], three lineages that are grouped together in the eukaryotic 'supergroup' Plantae (or Archaeplastida). Less clear is the number of times that photosynthesis has spread horizontally across the eukaryotic tree by 'secondary' endosymbiosis, a process whereby a primary-plastid-containing alga is taken up and assimilated by a second, non-photosynthetic host eukaryote [5]. Reasonable estimates range from as few as three to as many as seven (or more) distinct secondary endosymbioses involving both green and red algal endosymbionts. Remarkably, several clear examples of tertiary endosymbiosis have also been documented, in which a secondary-plastid-containing alga is engulfed by a distantly related eukaryote [5].

Secondary-plastid-containing algae are among the most genetically and morphologically diverse phototrophs on the planet: figuring out how they are related to one another by comparing the sequences of their genomes is anything but trivial. Secondary plastids are also patchily distributed across the eukaryotic tree, with the organisms that bear them often being most closely related to non-photosynthetic, plastid-lacking lineages. This is the situation with ciliates. These organisms belong to the alveolates, a lineage that also includes the red-tide-causing dinoflagellate algae and apicomplexan parasites [6]. Although exclusively non-photosynthetic, many apicomplexans (e.g., *Plasmodium* and *Toxoplasma*) possess a remnant plastid of red-algal origin retained for the purpose of fatty acid and isoprenoid biosynthesis [3]. The peridinin-pigmented plastids of dinoflagellates are also derived from red algae. To explain the origin of apicomplexan

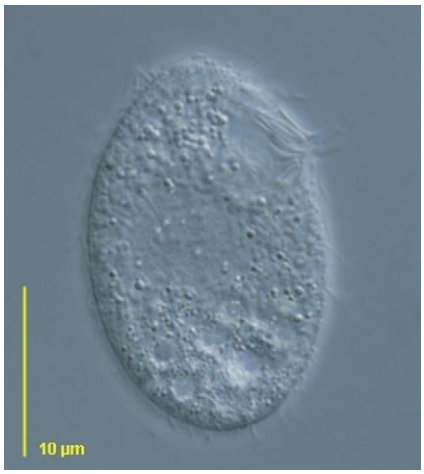

Figure 1. Light micrograph of *Tetrahymena*. Image by D. Patterson, L. Amaral-Zettler, M. Peglar and T. Nerad, provided with permission by http://microscope.mbl.edu.

and dinoflagellate plastids, one can either evoke independent secondary endosymbioses or a single endosymbiotic event in the dinoflagellate–apicomplexan common ancestor, coupled with subsequent plastid loss in at least some members of both lineages.

Toward the goal of further minimizing the number of secondary endosymbioses needed to account for the known distribution of plastids — and the complex genetic and cellular retooling that accompanies each event — Cavalier-Smith [7] proposed that a single, ancient endosymbiosis occurred in the common ancestor of all modern-day lineages harboring a red secondary plastid as well as their specific relatives, i.e., the 'chromists' (cryptophytes, haptophytes and photosynthetic stramenopiles), non-photosynthetic stramenopiles and alveolates — ciliates included. This so-called 'chromalveolate' hypothesis is arguably the most controversial idea of the last decade in the literature on plastid evolution (see [8] and references therein for recent reviews). While parsimonious from the perspective of plastid gain, the chromalveolate hypothesis demands that photosynthesis has been lost on numerous occasions, something that does not sit well with many researchers in the field. Nevertheless, support for this notion has come via direct or indirect evidence for vestigial plastids in several dinoflagellates once assumed to be ancestrally non-photosynthetic (e.g., [9,10]). The apicomplexan *Cryptosporidium* and the stramenopile *Phytophthora* are particularly interesting examples: neither possess a plastid but dozens of genes of putative cyanobacterial/algal origin reside in their nuclear genomes and have been interpreted as 'footprints' of a plastid-bearing phase in their evolutionary past [11,12]. Until recently, the ciliates were considered 'missing data'.

When the macronuclear genome of the model ciliate *Tetrahymena thermophila* (Figure 1) was published in 2006 [13], those with an interest in the evolutionary history of plastids paid close attention. Would genetic vestiges of a photosynthetic past be found? The initial search was negative. Not to be discouraged, Reyes-Prieto *et al.* [4] recently revisited the issue armed with an expanded taxonomic sampling and a new tree-topology-search tool (PhyloSort) geared to identify phylogenetic trees in which the ciliate homologs were monophyletic with 'chromalveolates' and/or members of the Plantae. Starting with 27,446 predicted *T. thermophila* proteins, a set of 3,997 candidate proteins was identified by reciprocal BLAST analysis and put through a phylogenomics pipeline, resulting in 133 trees that were subject to more rigorous analysis. In the end, the authors identified 16 protein trees with 'moderate to high' statistical support uniting the ciliates *T. thermophila* and *Paramecium tetraurelia* with the Plantae and, in most cases, at least some chromalveolates [4]. At face value, the results are potentially very exciting for chromalveolate enthusiasts, as they are consistent with a plastid-bearing phase at some point in the evolutionary history of ciliates. However, as Reyes-Prieto *et al.* [4] are quick to point out, the phylogenetic distribution and predicted functions of these 'algal' genes are complex and open to interpretation.

An alternative hypothesis is that some or all of the 16 algal genes in ciliates are not the remnants of past endosymbiotic gene transfer but are instead the product of horizontal gene transfer (HGT). The idea that HGT has been a significant factor in the evolution of eukaryotic genomes is gaining increasing acceptance [14]. In the case of ciliates, a recent expressed sequence tag-based study of the genomes of anaerobes living in the foregut of ruminants identified numerous cases of bacteria-derived genes involved in the breakdown of carbohydrates [15], indicating that ciliates can and do acquire genes from their prey. Indeed, some freshwater ciliates are known to harbor algal symbionts (e.g., [16]) and it will thus be important to determine the extent to which the putative algal genes in ciliates stand out against the background of HGTs in the *T. thermophila* and *P. tetraurelia* genomes. Nevertheless, the results of Reyes-Prieto *et al.* [4] provide an important launch point for further investigation of the origin of alga-derived genes in a more diverse array of ciliates and other chromalveolates.

It is important to consider that, even if the ciliates are shown to have evolved from photosynthetic ancestors, it does not mean that the chromalveolate hypothesis, as originally stated [7], is correct. This is because chromalveolates as a whole do not form a monophyletic assemblage in phylogenetic analyses, even those analyzing hundreds of nuclear genes (e.g., [17]). Complex models of plastid evolution in which a combination of secondary and tertiary endosymbioses are invoked to explain the apparent incongruence between 'chromalveolate' plastids and their host cells are gaining popularity [8].

At the same time as analyses of existing genomes continue to change our views on the deep evolutionary history of plastids, new organisms are being added to the mix. Perhaps most exciting is the recently discovered free-living alga *Chromera velia*, whose chlorophyll-*a*-containing plastids are of red-algal ancestry [18]. Most unexpectedly, plastid and nuclear ribosomal DNA gene phylogenies suggest that *C. velia* is more closely related to apicomplexan parasites than to any other phototrophic group, providing an intriguing link between the remnant plastids of apicomplexans and the peridinin-type plastids of dinoflagellates. The nuclear genome of *C. velia* holds great promise as a surrogate in global eukaryotic phylogenies for the derived and compositionally biased genomes of apicomplexans, and as an additional source of endosymbiotically derived genes for comparison to those predicted to exist in ciliates and other non-photosynthetic chromalveolates. Additional new and potentially

significant lineages include the katablepharids, which are non-photosynthetic (and apparently plastid-lacking) microbial eukaryotes linked to cryptophyte algae on the basis of molecular and morphological data [19], as well as the 'picobiliphytes', an as-yet uncultured photosynthetic lineage with plastids that are suspiciously cryptophyte-like in nature [20]. While it will likely be some time before their secrets are fully revealed, these important new pieces of the plastid evolution puzzle are definitely worth waiting for.

References

1. McGrath, C.L., Zufall, R.A., and Katz, L.A. (2006). Cilate genome evolution. In Genomics and Evolution of Microbial Eukaryotes, D. Bhattacharya and L.A. Katz, eds. (Oxford: Oxford University Press), pp. 64–77.
2. Greider, C.W., and Blackburn, E.H. (1985). Identification of a specific telomere terminal transferase activity in *Tetrahymena* extracts. Cell *43*, 405–413.
3. Waller, R.F., and McFadden, G.I. (2005). The apicoplast: a review of the derived plastid of apicomplexan parasites. Curr. Issues Mol. Biol. *7*, 57–79.
4. Reyes-Prieto, A., Moustafa, A., and Bhattacharya, D. (2008). Multiple genes of apparent algal origin suggest ciliates may once have been photosynthetic. Curr. Biol. *18*, 956–962.
5. Reyes-Prieto, A., Weber, A.P., and Bhattacharya, D. (2007). The origin and establishment of the plastid in algae and plants. Annu. Rev. Genet. *41*, 147–168.
6. Wolters, J. (1991). The troublesome parasites: molecular and morphological evidence that Apicomplexa belong to the dinoflagellate-ciliate clade. Biosystems *25*, 75–84.
7. Cavalier-Smith, T. (1999). Principles of protein and lipid targeting in secondary symbiogenesis: euglenoid, dinoflagellate, and sporozoan plastid origins and the eukaryote family tree. J. Eukaryot. Microbiol. *46*, 347–366.
8. Sanchez-Puerta, M.V., and Delwiche, C.F. (2008). A hypothesis for plastid evolution in chromalveolates. J. Phycol., in press.
9. Teles-Grilo, M.L., Tato-Costa, J., Duarte, S.M., Maia, A., Casal, G., and Azevedo, C. (2007). Is there a plastid in *Perkinsus atlanticus* (Phylum Perkinsozoa)? Eur. J. Protistol. *43*, 163–167.
10. Slamovits, C.H., and Keeling, P.J. (2008). Plastid-derived genes in the non-photosynthetic alveolate *Oxyrrhis marina*. Mol. Biol. Evol. *25*, 1297–1306.
11. Huang, J., Mullapudi, N., Lancto, C.A., Scott, M., Abrahamsen, M.S., and Kissinger, J.C. (2004). Phylogenomic evidence supports past endosymbiosis, intracellular and horizontal gene transfer in *Cryptosporidium parvum*. Genome Biol. *5*, R88.
12. Tyler, B.M., Tripathy, S., Zhang, X., Dehal, P., Jiang, R.H., Aerts, A., Arredondo, F.D., Baxter, L., Bensasson, D., Beynon, J.L., *et al*. (2006). *Phytophthora* genome sequences uncover evolutionary origins and mechanisms of pathogenesis. Science *313*, 1261–1266.
13. Eisen, J.A., Coyne, R.S., Wu, M., Wu, D., Thiagarajan, M., Wortman, J.R., Badger, J.H., Ren, Q., Amedeo, P., Jones, K.M., *et al*. (2006). Macronuclear genome sequence of the ciliate Tetrahymena thermophila, a model eukaryote. PLoS Biol. *4*, e286.
14. Andersson, J.O. (2005). Lateral gene transfer in eukaryotes. Cell. Mol. Life Sci. *62*, 1182–1197.
15. Ricard, G., McEwan, N.R., Dutilh, B.E., Jouany, J.P., Macheboeuf, D., Mitsumori, M., McIntosh, F.M., Michalowski, T., Nagamine, T., Nelson, N., *et al*. (2006). Horizontal gene transfer from Bacteria to rumen Ciliates indicates adaptation to their anaerobic, carbohydrates-rich environment. BMC Genomics *7*, 22.
16. Summerer, M., Sonntag, B., and Sommaruga, R. (2008). Ciliate-symbiont specificity of freshwater endosymbiotic *Chlorella* (Trebouxiophyceae, Chlorophyta). J. Phycol. *44*, 77–84.
17. Burki, F., Shalchian-Tabrizi, K., and Pawlowski, J. (2008). Phylogenomics reveals a new 'megagroup' including most photosynthetic eukaryotes. Biol. Lett. *4*, 366–369.
18. Moore, R.B., Obornik, M., Janouskovec, J., Chrudimsky, T., Vancova, M., Green, D.H., Wright, S.W., Davies, N.W., Bolch, C.J., Heimann, K., *et al*. (2008). A photosynthetic alveolate closely related to apicomplexan parasites. Nature *451*, 959–963.
19. Okamoto, N., and Inouye, I. (2005). The katablepharids are a distant sister group of the Cryptophyta: A proposal for Katablepharidophyta Divisio Nova/ Katablepharida Phylum Novum based on SSU rDNA and beta-tubulin phylogeny. Protist *156*, 163–179.
20. Not, F., Valentin, K., Romari, K., Lovejoy, C., Massana, R., Tobe, K., Vaulot, D., and Medlin, L.K. (2007). Picobiliphytes: a marine picoplanktonic algal group with unknown affinities to other eukaryotes. Science *315*, 253–255.

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Sleep: Approaching the Fundamental Questions

Our 'sleep need' may be defined as the daily amount of sleep we require to be fully awake during daytime. A recent study has shown that both sleep need and daytime sleepiness decline in older adults.

Christian R. Baumann

> "*Try and penetrate with our limited means the secrets of nature, and you will find that, behind all the discernible concatenations, there remains something subtle, intangible and inexplicable*."
>
> — Albert Einstein (1879–1955)

Within the last few years, a number of important discoveries have been made in the field of sleep medicine, leading to a better understanding of the regulation of sleep and wakefulness. But some fundamental questions remain unanswered, such as: Why do we sleep? What are the functions of deep sleep and of rapid eye movement sleep? And how much sleep do we really need?

As they report in this issue of *Current Biology*, Klerman and Dijk [1] have met the challenge posed by this last question, and give the most comprehensive answer to date. They examined sleep and wakefulness in healthy young (18–32 years) and older (60–76 years) subjects, both under habitual circumstances (at home), and in the sleep laboratory, by minimizing confounding social and circadian influences and offering extended sleep opportunities. To examine the maximal capacity for sleep, the researchers encouraged the subjects to sleep up to 16 hours per day. They made two important observations. First, that increasing age is accompanied by a reduction in sleep need. The total sleep duration per 24 hours was 1.5 hours shorter in the older subjects (7.4 *versus* 8.9 hours). Both rapid eye movement sleep and non-rapid eye movement sleep were reduced in older subjects. Second, that habitual sleep time (at home) was not different between young and older subjects, but young subjects more often suffered from excessive daytime sleepiness. This finding suggests that young subjects are either not able or do not allow themselves to sleep enough.

How much sleep do we really need? There is considerable evidence of a continuous reduction in average sleep time by 20% over the past century [2]. Sleep need may be defined